

Study on Measurement Method of Anaerobic Sludge Activity: Hydrogen Production

Yage Y¹, Rui X^{1*}, Jianchang L¹, Huanyun D¹, Qiuxia W¹ and Hadi N²

¹College of Energy and Environment Sciences, Solar Energy Institute, Yunnan Normal University, Kunming, China

²CEE Department, Earth and Environmental Sciences Lab Division, UC Berkeley, Berkeley, CA, USA

Abstract

In this research finding, a new method is derived by adopting Maximum Specific Hydrogen Production Rate ($U_{\max} \cdot H_2$) to measure the activities of anaerobic sludge for hydrogen production. With batch fermentation instrument under mesophilic ($35^\circ\text{C} \pm 1^\circ\text{C}$) condition the research has been implemented and concluded. In this study, during the demonstration of experiments, for hydrogen fermentation as for the inoculums there three different active sludges are used and as for the feeding materials there kitchen wastes are tested. After resulting analysis it's been showed that using the Maximum Specific Hydrogen Production Rate ($U_{\max} \cdot H_2$) as a means to be an index or a method of sludge activity measurement-is reported as satisfactorily feasible and applicable. The $U_{\max} \cdot H_2$ values of experimental groups for this research methodology are recorded as the following: $U_{\max} \cdot H_2(A)=30.24\text{mL/gVSS} \cdot \text{h}$, $U_{\max} \cdot H_2(B)=10.80\text{mL/gVSS} \cdot \text{h}$, $U_{\max} \cdot H_2(C)=18.05\text{mL/gVSS} \cdot \text{h}$. The Correlations between $U_{\max} \cdot H_2$ and other parameters, such as cumulative hydrogen yield, fermentation period and degradation rate of TS are all remain Significant throughout the research. During experimental implementation, Pearson Correlation between $U_{\max} \cdot H_2$ and fermentation period is reported as 0.997 achieving statistical Significance 0.047 (<0.05). Pearson Correlation between $U_{\max} \cdot H_2$ and cumulative hydrogen yield is reported as 0.999 achieving a considerable trend toward Significance 0.022 (<0.05). Pearson Correlation between $U_{\max} \cdot H_2$ and degradation rate of TS is reported as 0.999 Signifying a marginal trend toward Significance 0.027 (<0.05).

Keywords: Maximum specific hydrogen production rate; Kitchen waste; Anaerobic sludge activity; Hydrogen production; Anaerobic inoculums; Degradation rate

Introduction

With increasing number of population and associated people's living standard-have been causing the rapid growth and production of kitchen wastes in current ages. It is well estimated that 36.5 million tons of kitchen wastes are being produced each year in India [1]. Even in China, 1.61 million tons of family food wastes had been being produced until 2013 [2]. Kitchen wastes are unquestionably harmful and can be active factors of environmental pollution being infectious to human health if its' not been treated promptly and properly-because of the remaining or left over wastes contain high contents of water and organic materials [3], which are certainly putrefactive [4] by causing groundwater resources polluted. Emission of Volatile Organic Sulfur Compounds (VOCs) during aerobic decomposition of food wastes can cause air pollution [5]. All sorts of pollutions can be promoted and produced during transforming food residue to feed [6]. As known kitchen waste contains a lot of *Salmonella*, *Staphylococcus aureus* and other pathogenic microorganisms [7], which can be inimitably dangerous to peoples' health. Therefore, it has become a worldwide catchy matter of concern that how to make the food waste harmless, recycled and reproduced economically and sustainably. The common processing methods for kitchen wastes can be as reported from previous works are-direct emissions after mechanical pulverization [8], incineration and landfill [8], usage for feeding [9], composting [10], anaerobic digestion technology [11], etc. To treat the kitchen waste using anaerobic digestion technology can be beneficiary of both the elimination process of environment pollution and the resource utilization of wastes. Hydrogen production is one of the effective and efficient applications of anaerobic digestion technologies, which is preceded by physiological and metabolic activity of microbial. By dehydrogenation of organic compounds in the fermentation process, the surplus electrons of the redox process are balanced to ensure the smooth metabolic processes for the hydrogen production and inspection [12,13].

The choice of activated sludge in anaerobic digestion for hydrogen production plays an important role. At present, the indexes of activated sludge usually include Dehydrogenase Activity, ATP content, Oxygen Up taking Rate, Mixed Liquor Suspended Solid (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS), Sludge Volume Index (SVI), Headspace Gas Chromatography Technology, Maximum Specific Methane Production Rate, etc. [14-18]. However, most of the above mentioned methods are mainly designed by concentrating on the activity measurement of the aerobic sludge; which clearly states that the study on fermentative activity measurement of anaerobic sludge is insufficient and not even on consideration table in recent research works. In this research article, $U_{\max} \cdot H_2$ is defined as a measurement method of anaerobic sludge activity, and three different inoculums are cultivated and are adopted to test the measurement method to initiate the hydrogen production process of kitchen wastes. The study is to provide certain scientific basis for screening and preventing the demonstration of highly activated sludge, hydrogen production by anaerobic fermentation and energy-oriented use of kitchen wastes.

Materials and Methods

Feeding material

The feeding materials for this study are the kitchen wastes, which are taken from the canteen of Yunnan Normal University. After picking

*Corresponding author: Rui X, College of Energy and Environment Sciences, Solar Energy Institute, Yunnan Normal University, Kunming, 650092, China, Tel: +8613888083229; E-mail: ecowatch_xr@163.com

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and separating out the plastic bags, chopsticks and other undigested sundries, the residues are dried out using thermostat oven at 105°C and pulverized into powder (30-200 mesh) using the pulverizer (Shanghai Guning Instrument Co., Ltd., DFY-400C, 30-200 mesh). The powder materials are directly used as feeding materials of digestion measurement. The Total Solids (TS) and Volatile Solids (VS) of feeding materials are measured before and after the fermentation process.

Inoculums

The inoculums A and C are obtained from the Biomass Energy Laboratory of Solar Energy Institute, Yunnan Normal University. Inoculums B is the sludge that has been obtained from a wastewater treatment plant in Kunming, China. The methods of the inoculums cultivation are captivated from Enrichment Culture [19]. To carry out the cultivation process-the substrate and fermentation residue are to be mixed at the ratio of 3:2 [20,21] then the mixture is to be placed in a sealed container to cultivate for 1-2 months until it turns in color of black or gray-black and gas production comes to the stop [22,23]. Inoculums A is to be cultivated at 35°C, and inoculums C is to be cultivated at ambient temperature.

Experimental design

In this finding, Batch fermentation technique [24] is adopted as the designing process of experiment, and all the experiments are carried out under mesophilic (35°C ± 1°C) condition. Three experimental groups and three parallels for each group are set to run the experiment all through.

The quantity of waste and inoculums are calculated by the following formula [25].

$$W_0 \times C_{TS} = W_1 \times TS_1 + W_2 \times TS_2 \quad (1)$$

In which, C_{TS} is the TS (%) of the fermentation mixture; W₀ is the total amount (g) of the fermentation mixture; W₁ is the feeding amount (g) of wastes; W₂ is the feeding amount (g) of the inoculums; TS₁ is the TS (%) of wastes; TS₂ is the TS (%) of inoculums.

Experimental equipment

The experiment equipment (Figure 1) consists of a digestion bottle, a gas collecting bottle, a volume measurement bottle and a set of temperature control device.

The capacity of fermentation system is 500 ml, and the air tightness of system is checked before the fermentation process in order to avoid possible gas leakage. The produced gas does get into the gas collecting bottle from fermentation bottle; and which then form the saturated NaHCO₃ solution into volume measurement bottle from the gas collecting bottle. The volume of the production gas can be obtained by reading the scale of measurement bottle.

Measurement method

During the experimental installation and implementation, the TS and VS have been measured by the standard analysis methods [26].

pH value: Precision acidity meter of PHS-3C (Shanghai Hongyi Instrumentation Co., Ltd., 04C-482) is to measure the value. The VSS is measured by the gravimetric method [27].

Gas yield [28] using NaHCO₃-draining (saturated solution of NaHCO₃) method is to record the daily gas yield of each group in regular basis right after the experiment starts. In each group, during the field study as the characteristic gas yield, the average gas yield of 3 parallels is assumed.

Hydrogen concentration: Using Gas Chromatograph (Lunan Analytical Instrument Co., Ltd, GC-6890A) hydrogen concentration is to be measured.

Statistical method: Statistical analysis of Data using software named of SPSS and Origin 8.6.

Analysis method

The maximum specific hydrogen production rate (U_{max}•H₂) is defined as the maximum yield of hydrogen gas per h per gram of VSS (indirectly represents biomass living weight of anaerobic sludge), the unit is mL/g•VSS•h.

The following expression can be drawn from the Monod equation $U = U_{max} \cdot S / (K_s + S)$:

$$\frac{dS}{dt} = -\frac{U_{max} \cdot SX}{K_s + S} \quad (2)$$

In the formula, S: Substrate concentration (gVS/L); t: time (h); U_{max}: Substrate maximum degradation rate (h⁻¹); SX: Saturated microorganism concentration (gVSS/L); K_s: Saturation constant (g/L). The net sludge yield coefficient is declared as in small number under anaerobic condition. Concentration of microbes (recording it as X) is high in the reactor, while microbiological increment (recording it as ΔX) is much smaller than X. Therefore, X is considered to be approximately constant. In the measurement area, dissolved gas in the mixture is saturated; the gas dissolved in the mixture can be neglected. The kitchen waste is hydrolyzed fast in anaerobic systems, hydrogen production rate of kitchen waste is proportional to the rate of matrix degradation, and the following equation can be deduced as follow:

$$\frac{dVH_2}{dt} = -Y_g V_R \frac{ds}{dt} \quad (3)$$

In which, VH: Cumulative production of hydrogen (mL); Y_g: Conversion coefficient from the substrate to the gas (mLgas/gVSSsubstrate); V_R: Reaction zone volume (L).

From the above equation, it can be deduced as follow:

$$\frac{dVH_2}{dt} = \frac{Y_g V_R U_{max} SX}{K_s + S} \quad (4)$$

At the beginning of the reaction, substrate concentration is high, S >> K_s, K_s is negligible. Equation (4) can be as follow:

$$\frac{dVH_2}{dt} = Y_g V_R U_{max} X, \text{ making } U_{max} \cdot H_2 = Y_g U_{max}.$$

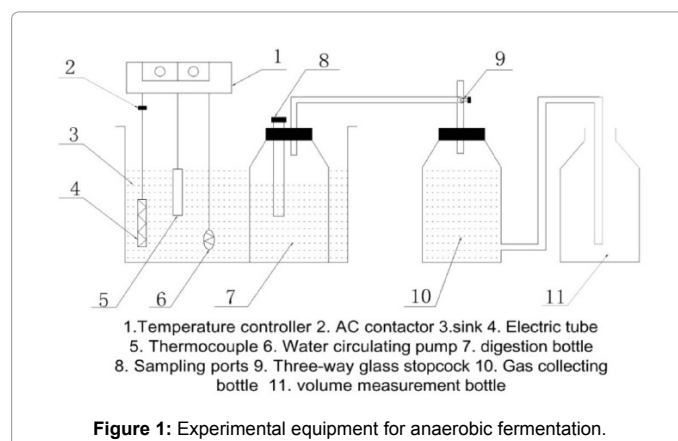


Figure 1: Experimental equipment for anaerobic fermentation.

Thus the maximum specific hydrogen production rate can be obtained as follow:

$$U_{max} \cdot H_2 = \frac{1}{V_r X} \cdot \frac{dV_H}{dt} \quad (5)$$

According to the linear regression equation which is obtained in measurement area [29-31] from which the slope of the linear regression equation $\frac{dV_H}{dt}$ can be calculated (Table 1).

Based on the statistical principle, multiple comparisons have been used to analyze the mean difference of fermentation indexes between the experimental groups. And the correlation between $U_{max} \cdot H_2$ and other indexes have been analyzed in order to discuss and represent that whether the use of $U_{max} \cdot H_2$ as a measurement method of sludge activity is feasibly demonstrative or not. Fermentation indexes are selected as follows in order to show the hydrogen production activity of sludge [32-34] the maximum hydrogen production per h is used to mirror the peak of hydrogen production; the fermentation period is used to show the total time consumption in fermentation process, the cumulative hydrogen yield is used to realize the potential of the hydrogen production, the degradation rate of TS is used to reflect the material consumption.

Results and Discussion

Analysis results of relevant indexes of fermentation

As shown in Table 2, the TS and VS value of fermentation liquid in experimental groups get reduced after the fermentation process, and the pH value gets increased considerably. It can be considered and explained as that the raw materials are consumed to produce hydrogen and organic acids. And the depletion of organic acids makes the pH value increased. The TS and VS consumption ratios of experimental group A are obtained in amount of 27.43% and 30.40% respectively. The TS and VS consumption ratios of experimental group B are found in rate of 11.20% and 14.61% respectively. TS and VS consumption ratios of experimental group C are recorded in rate of 16.64% and 19.56% respectively. It represents the little change about the indicators of the control groups.

Analysis of hydrogen net production curve

Figures 2 and 3 shows that the hydrogen net production (the experimental group production value minus the control group

Item	TS (%)	VS (%)	VSS(g/L)	pH Value
Powder Wastes	97.62	88.85	-	-
Inoculum A	4.62	69.27	4.84	5.09
Inoculum B	9.57	66.44	6.21	5.03
Inoculum C	7.56	69.70	4.52	4.91

Table 1: TS, VS and pH values of original materials and inoculums.

Item	Before Fermentation			After Fermentation		
	TS (%)	VS (%)	pH Value	TS (%)	VS (%)	pH Value
Control Group A	2.55	68.6	5.12	2.33	66.47	5.19
Control Group B	3.05	66.24	5.07	2.96	65.78	5.11
Control Group C	3.69	67.69	4.96	3.44	66.78	5.02
Experimental Group A	6.78	88.82	4.62	4.92	85.19	5.41
Experimental Group B	6.43	77.44	4.56	5.71	74.46	5.49
Experimental Group C	6.97	82.3	4.58	5.81	79.42	5.37

Table 2: TS, VS and pH value of feed liquid.

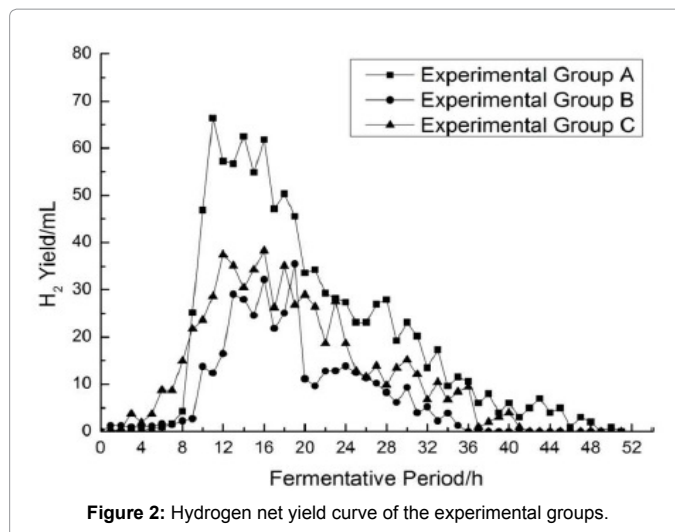


Figure 2: Hydrogen net yield curve of the experimental groups.

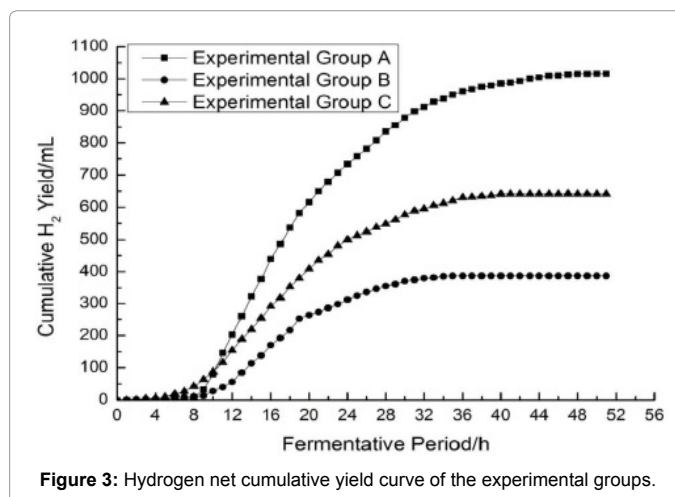


Figure 3: Hydrogen net cumulative yield curve of the experimental groups.

production value) per h is not similar, hydrogen net production that do differ at every experimental group are as follows: In group A, hydrogen production gets increased rapidly after 9th h, and its' peak-hour is found in 10th-19th h. The hydrogen yield is reduced after 20th h. In-group B; hydrogen production begins to increase after 10th h. Its' peak-h is found in 13th-19th h. The hydrogen yield gets reduced after 20th h. In-group C, hydrogen production gets increased rapidly after 8th h, whose peak-h is listed at 10th-24th h. The hydrogen yield is reduced after 25th h.

Calculation and analysis on the activity of hydrogen production

As shown in Figures 4-6 the slope of the regression equation which are obtained from the measurement area [29-31] of cumulative gas production curve of experimental group A, B, C are: KA=58.56; KB=26.82; KC=32.63. And thus the $U_{max} \cdot H_2$ could be obtained by the calculation.

As shown in Table 3, $U_{max} \cdot H_2(A) > U_{max} \cdot H_2(C) > U_{max} \cdot H_2(B)$, which means that the activities of the inoculums (A, B and C) in the fermentation process of the kitchen wastes to produce hydrogen are different. Statistical result shows that the difference of activities (inoculums A, B and C) is considerably Significant (Sig.<0.01).

In startup phase of reaction, the substrate is enough to run the experiment, and Michaelis-Menten equation shows that the reaction is

Experimental Group	Maximum Hydrogen Production Per Hour (MHPH)/ml	Fermentation Period (FP)/h	Cumulative Hydrogen Yield (CHY)/ml	Degradation Rate of TS (DR)/%	$U_{max} \cdot H_2$ mL/gVSS·h
A	66.00 ^a	51.00 ^a	1016.00 ^a	27.43 ^a	30.24 ^a
B	35.00 ^b	35.00 ^b	387.00 ^b	11.20 ^b	10.80 ^b
C	38.00 ^b	42.00 ^c	641.00 ^c	16.64 ^c	18.05 ^c

Table 3: Indexes of anaerobic fermentation.

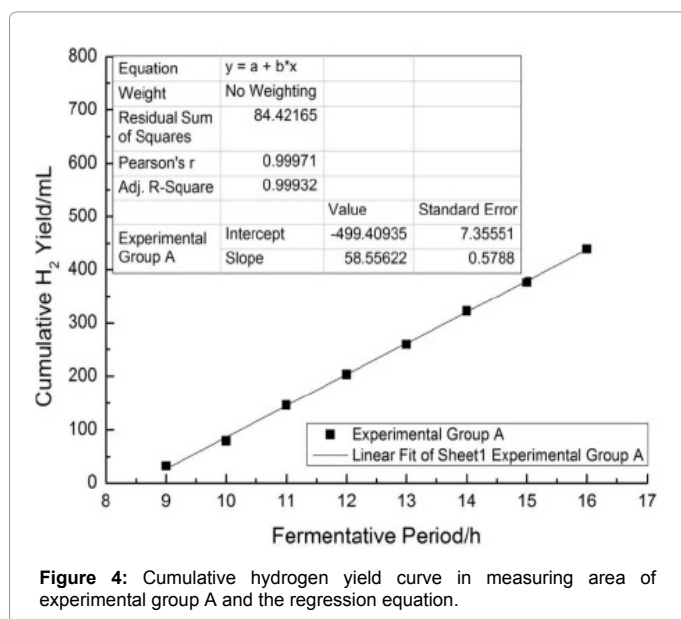


Figure 4: Cumulative hydrogen yield curve in measuring area of experimental group A and the regression equation.

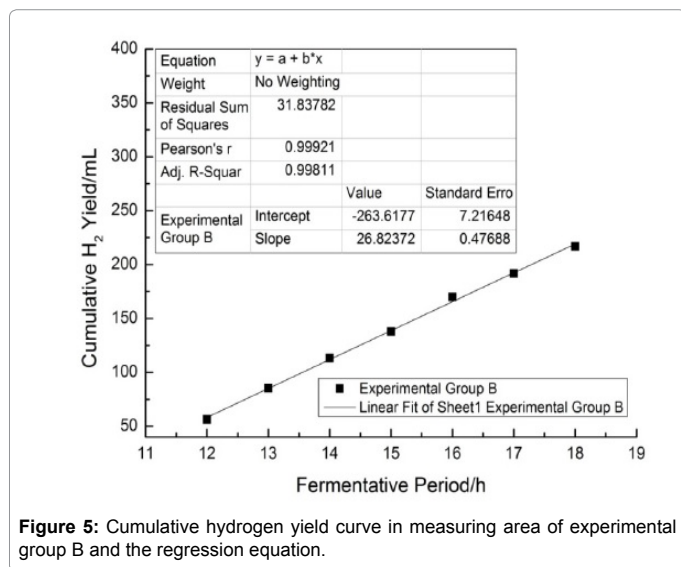


Figure 5: Cumulative hydrogen yield curve in measuring area of experimental group B and the regression equation.

in zero order in a period of time, the Specific Hydrogen Production Rate is constant, and its numerical value is equal to the Maximum Specific Hydrogen Production Rate. $U_{max} \cdot H_2$ shows as constant, it represents that the maximum capacity of anaerobic microbial degradation of the organic matrix. For the same substrate, having the higher $U_{max} \cdot H_2$ value can result in the activity of the anaerobic sludge even as stronger. $U_{max} \cdot H_2$ can be used to compare the activity of different anaerobic sludges in the same environmental conditions, or compare the activity of anaerobic sludge in different environmental (such as in different temperatures) conditions. It can also be used to evaluate the ability of anaerobic sludge to the degradation process of different substrates.

Analysis on the indexes of anaerobic fermentation

Table 3 indicates that indexes of experimental group A are better than the other experimental groups. Statistical result shows that the Mean Difference of fermentation period, cumulative hydrogen yield, degradation rate of TS and $U_{max} \cdot H_2$ is considerably significant (Sig.<0.01) in different experimental groups. It illustrates that the activities of three inoculums in the fermentation process of the kitchen wastes to produce hydrogen are remarkably different. $U_{max} \cdot H_2$ can be used as an activity index of sludge for the hydrogen production, and the obtained results from it are consistent with other fermentation indexes.

As shown in Table 4, the Correlations between $U_{max} \cdot H_2$ and other parameters-such as cumulative hydrogen yield, fermentation period and degradation rate of TS are all significantly identical up to 95% level. It indicates that using $U_{max} \cdot H_2$ as an index of fermentation for hydrogen is feasible. In addition, highly active sludge can be screened through this method.

The Mean Difference of maximum hydrogen production per h is not significant between experimental group B and group C (Sig.>0.01). And the Pearson Correlations between maximum hydrogen production per h and other indexes are not significant (Sig.>0.05). It indicates that using maximum hydrogen production per h as a sole index of fermentation process is not recommendable and feasible.

Conclusion

- From the statistical results of the experimental data it is showed that $U_{max} \cdot H_2$ of three experimental groups are found different from each other. The mathematical representation of the results of researched activities can be stated as following: $U_{max} \cdot H_2(A) > U_{max} \cdot H_2(C) > U_{max} \cdot H_2(B)$.
- The indexes of three different inoculums for hydrogen production using anaerobic fermentation are found different from each other and the Mean Difference is reported as Significant (Sig.<0.01) where there it's been found that the group experimental A gets the highest production rate than the followed experimental group B and the experimental group C, also listed that experimental group B has the lowest production rate.
- By the Correlation analysis between $U_{max} \cdot H_2$ and other fermented indexes its' been showed that using $U_{max} \cdot H_2$ as an index or method of the activity of inoculums for hydrogen production is practically feasible and implementable, and the prominent and satisfactory value of which can be obtained by running the experiment as discussed.

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Item		$U_{max} \cdot H_2$	Maximum Hydrogen Production Per Hour	Fermentation Period	Cumulative Hydrogen Yield	Degradation Rate of TS
$U_{max} \cdot H_2$	Pearson Correlation	1	0.958	0.997	0.999	0.999
	Significant		0.185	0.047	0.022	0.027
Maximum Hydrogen Production Per Hour	Pearson Correlation	0.958	1	0.935	0.948	0.969
	Significant	0.185	-	0.232	0.207	0.158

Table 4: Correlation between different indexes.

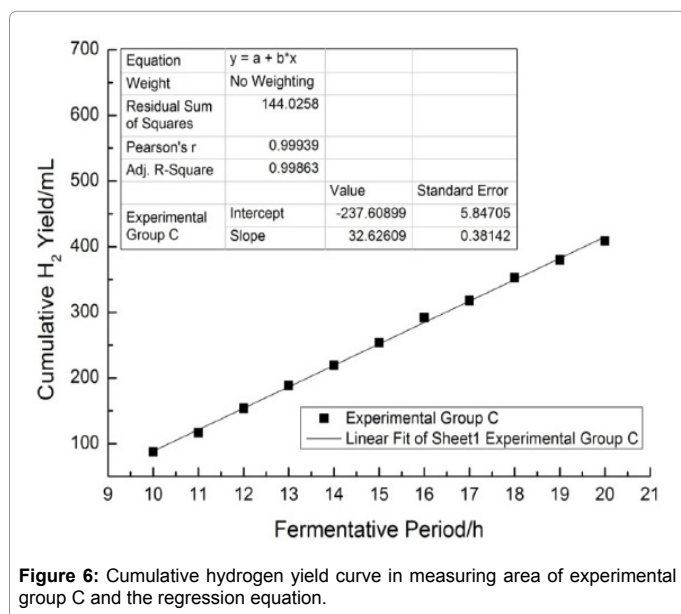


Figure 6: Cumulative hydrogen yield curve in measuring area of experimental group C and the regression equation.

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